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Sex Pheromone of the Cranberry Root Grub *Lichnanthe vulpina*

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Abstract The cranberry root grub *Lichnanthe vulpina* (Hentz) (Coleoptera: Glaphyridae) is a pest of cranberries in Massachusetts, reducing yield and vine density. (*Z*)-7-Hexadecenol and (*Z*)-7-hexadecenal were identified from the female effluvia collection by gas chromatographic–electroantennographic detection and gas chromatography–mass spectrometry. The double-bond position was confirmed by dimethyl disulfide derivatization. Both compounds were tested in the field, each alone and as blends of the two. Each compound alone captured males; however, (*Z*)-7-hexadecenol alone captured significantly more males than did (*Z*)-7-hexadecenal alone. The addition of varying amounts of (*Z*)-7-hexadecenal to (*Z*)-7-hexadecenol did not statistically affect male capture. Flight activity of the cranberry root grub may be monitored with traps baited with rubber septa containing 300 μg of (*Z*)-7-hexadecenol. A test of trap vane colors indicated that traps with green or black vanes maximized target male catch while minimizing nontarget catch of important cranberry pollinators.

Keywords (*Z*)-7-Hexadecenol · (*Z*)-7-Hexadecenal · Gas chromatography–mass spectrometry · Electroantennogram · Scarab beetle · Glaphyridae

*Deceased May 15, 2001. He is dearly missed by his family, friends, and colleagues.

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Introduction

The cranberry root grub (CRG) *Lichnanthe vulpina* (Hentz) (Coleoptera: Glaphyridae) is a native scarab beetle species whose immature stages have a long history as root-feeding pests of cranberry beds in Massachusetts, first being reported in 1911 (Franklin, 1950). Dunn and Averill (1996) found that of the 38 Massachusetts cranberry beds sampled, 61% were infested with CRG larvae. Currently, the only control strategy for the CRG is renovation. Bog renovation, the removal and disposal of the top 25–30 cm of the bog (including the cranberry plants and associated CRG larvae), is an extreme and expensive solution for a problem that may eventually recur. The identification and commercial availability of the CRG sex pheromone of this species could provide a useful tool for monitoring or managing this pest.

The objective of this study was to identify the sex pheromone of *L. vulpina* as well as to determine a trap color that would maximize target capture while minimizing capture of honeybees and bumblebees, important pollinators of cranberries.

For more information on the taxonomy, behavior, and distribution of the *Lichnanthe*, see Westcott (1976), Carlson (1980), and O'Donnell (1996).

Methods and Materials

Pheromone Collections

Third instar larvae of CRG were collected in mid-April by digging them from the soil in an infested cranberry bog in Carver, MA. Recovered larvae were kept individually in ~30-ml plastic cups in a 3:1 mix of greenhouse sand and screened peat moss raised to ca. 12% moisture. They were housed in a controlled environment room at 25°C during the 16-hr photophase and 20°C during the 8-hr scotophase. After pupation and adult emergence, individuals were separated by sex, and up to 15 females were placed together in an all-glass collection vessel during the photophase because the species is diurnal (Zhang et al., 1994; O'Donnell, 1996). Twelve female collections were made during the course of the study. During the photophase, pump-drawn air was filtered through charcoal, bubbled through distilled water, passed over and among the females, and finally through a glass tube filled with adsorbent Super Q polymer material (Alltech, Deerfield, IL, USA). Volatiles were eluted from the Super Q using ca. 2 ml of dichloromethane before condensing under a nitrogen stream to a volume of ca. 20 μl.

Instrumentation

The coupled gas chromatograph–electroantennogram detector (GC-EAD) system used was as previously described in Zhang et al. (1997, 1999, 2003). A Hewlett Packard (HP) 5880 gas chromatograph equipped with a 30 m × 0.25 mm ID, 0.25-μm film-thickness nonpolar SE-30 capillary column (Alltech Associates) in the splitless mode with nitrogen as carrier was used for GC-EAD analysis (150°C for 2 min, then programmed to 250°C at 10°C/min and held for 25 min) or a 30 m × 0.25 mm ID, 0.25-μm film-thickness polar Stabilwax capillary column (Restek Corp., 150°C for 2 min, then programmed to 220°C at 10°C/min and held for 25 min). The capillary column effluent and nitrogen makeup gas (10 ml/min) were split (~1:1) by a Y GlasSeal capillary column connector (Supelco, Inc.) to the flame ionization detector (FID) and EAD. After removing an antenna from the beetle, one lamella

tip and the scape were positioned between two gold wire electrodes, which were immersed in saline-filled (0.9% NaCl) wells in a small acrylic plastic holder. This holder held the antennal club open, exposing the sensilla to the airstream (see photo in Robbins et al., 2003). The output recording electrodes were connected to a high-impedance 1:100 amplifier with automatic baseline drift compensation. The airstream flowing over the antennae (about 500 ml/min) was humidified by bubbling through distilled water before entering the EAD interface. The antennal preparation was cooled to ~5°C inside a condenser by circulating near 0°C water from a bench-top refrigeration unit (RTE-100, NESLAB instruments, Inc., Portsmouth, NH, USA) through the insulation layer of the modified condenser containing the acrylic plastic holder mounted on top of the GC. An HP 3390A integrator was used for EAD recording.

Electronic impact gas chromatography–mass spectrometry (GC-MS) was conducted on an HP 5890 GC coupled to an HP 5970B mass selective detector using an identical SE-30 capillary column [150°C for 2 min, then programmed to 250°C at 10°C/min and held for 25 min for regular analysis; 180°C for 2 min, then programmed to 230°C at 15°C/min and held for 50 min for analysis of dimethyl disulfide (DMDS) adducts] or a DB-5 capillary column (60 m × 0.25 mm ID, 0.25-μm film thickness, J&W Scientific Inc.; 50°C for 2 min, then programmed to 300°C at 15°C/min and held for 50 min) but with helium as carrier gas. A 70-eV electron beam was employed for sample ionization.

Chemicals

Synthetic (*Z*)-7-hexadecenol (Z7-16:OH) and (*Z*)-7-hexadecenal (Z7-16:Ald) were purchased from Pheromone Bank, Wageningen, The Netherlands. Purities of the chemicals, as determined on 30-m polar Stabilwax and nonpolar SE-30 GC capillary columns, were > 98%.

Microderivatization

Dimethyl disulfide derivatives of extracts and synthetic standards were prepared according to standard procedures (Buser et al., 1983; Dunkelblum et al., 1985). Dichloromethane solutions of effluvia extracts or hexane solutions of synthetic monounsaturated standards (10 μl, 20 ng/μl) were treated with 50 μl of DMDS (Aldrich Chem. Co., 99+%) and one drop of an iodine solution (60 mg/ml diethyl ether). The mixtures were kept at 60°C for 4 hr. After cooling to room temperature, one drop of 5% aqueous sodium thiosulfate ($Na_2S_2O_3$) was added, and the solutions were shaken vigorously to reduce the iodine. The organic phase was removed, and the aqueous phase was extracted with 100 μl hexane. The combined extracts were then dried over anhydrous sodium sulfate (Na_2SO_4) and concentrated to ~20 μl under nitrogen for GC-MS analysis.

The effluvia collection, synthetic Z7-16:OH, and synthetic Z7-16:Ald (30 ng in hexane) were each treated separately in a conical glass vial with 5 μl of acetic anhydride–pyridine (10:1, v/v), sealed with a Teflon lined screw cap, and heated at 40°C in a GC oven for 30 min. After 1 μl water was added to destroy the excess anhydride, the organic layer was removed for GC-MS analysis.

Field Evaluation of Synthetic Lures

The following protocol was used for field testing in all years. Lures were formulated by dissolving Z7-16:OH and Z7-16:Ald in hexane, dispensing appropriate amounts into

5-mm rubber stopper septa (Thomas Scientific, Swedesboro, NJ, USA), and allowing the hexane to evaporate in a fume hood. Lures were deployed in the field in cross-vane traps. Traps were placed ca. 20 m apart along the cranberry bog edge and were randomized at deployment. The bottom of the trap was hung ca. 60 cm from the ground. Dates in parentheses at the end of each section indicate the dates that the traps were checked and rerandomized.

Effect of Different Proportions of the Pheromone Constituents on CRG Trap Catches

In 1999, treatments tested in the field included eight blends of Z7-16:OH and Z7-16:Ald at a dose of 1000 μg/septa in the ratios of 100:0, 90:10, 80:20, 60:40, 40:60, 20:80, 10:90, and 0:100 and a solvent-only control treatment. One set of the nine treatments was deployed at each of three Massachusetts cranberry bogs during the flight period in July (July 6–13, 15, 16, and 19).

Effect of Different Doses of a 90:10 Blend of the Alcohol and Aldehyde on Trap Catches

In 2000, a test was deployed to compare doses of a 90:10 blend of Z7-16:OH and Z7-16:Ald. The doses included 100, 300, 600, and 1000 μg/septa and a solvent-only control septa. One set of five treatments was deployed at each of four Massachusetts cranberry bogs during the flight period in July (July 3–9, 14, 18, and 25).

Effect of Trap Vane Color on CRG and Nontarget Insect Catches

A test was deployed in 2000 to test the effect of vane color on CRG adult male catch and honeybee and bumblebee catches. Honeybee and bumblebee catches are of concern to growers because they are essential for crop pollination. This test was conducted to determine a color that would maximize CRG male catches while reducing bee catches. All traps were baited with lures consisting of a 90:10 blend of Z7-16:OH and Z7-16:Ald at a dose of 1000 μg/septa. Vanes for the lab-constructed cross-vane traps were fabricated from 4-mm corrugated plastic of six different colors (white, red, yellow, blue, black, and green) purchased from KIVA Container Corporation, Taylors, SC, USA. One set of six treatments was deployed at each of four Massachusetts cranberry bogs during the flight period in July (July 3–9, 14, 18, and 25).

Effect of the Presence of Different Amounts of the Aldehyde on the Optimum Dose of the Alcohol on Male Catches

In 2004, a test was deployed to compare male CRG capture when various amounts of Z7-16:Ald were added to 1000-μg doses of Z7-16:OH. This test was initiated because the results of the 1999 test (see Fig. 2) did not resolve the role of Z7-16:Ald in male capture. The six treatments tested included 1000 μg of Z7-16:OH alone, 1000 μg of Z7-16:OH + 10% (111 μg) of Z7-16:Ald, 1000 μg of Z7-16:OH + 20% (250 μg) of Z7-16:Ald, 1000 μg of Z7-16:OH + 40% (666 μg) of Z7-16:Ald, 1000 μg of Z7-16:Ald, and a solvent-only control. One set of the six treatments was deployed at each of two Massachusetts cranberry bogs, and two sets were deployed at separate locations on a third bog during the flight period (June 28, July 1, 5, and 8).

In 2005, a test was deployed to compare male CRG capture when various amounts of Z7-16:Ald were added to 300-μg doses of Z7-16:OH. In this test, the dose of Z7-16:OH was

reduced from 1000 μg/septa (see 2004 test above) to 300 μg/septa to provide a more sensitive assay of male CRG response to added amounts of the Z7-16:Ald. The three treatments tested included 300 μg of Z7-16:OH alone, 300 μg of Z7-16:OH + 10% (33 μg) of Z7-16:Ald, and 300 μg of Z7-16:OH + 20% (75 μg) of Z7-16:Ald. Four sets of the three treatments were deployed at separate locations on a large Massachusetts cranberry bog during the flight period (July 3, 5, 8, 11, 16, and 19).

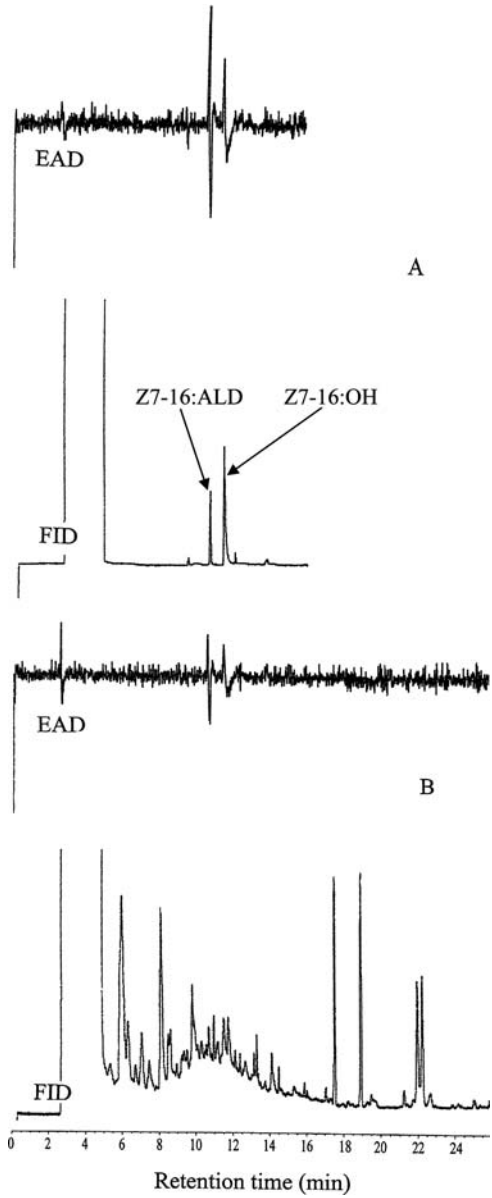

Fig. 1 Simultaneous EAD and FID responses of a male CRG antenna to (A) synthetic Z7-16:Ald and Z7-16:OH (10 ng, 1:3 ratio, v/v); (B) effluvia trapped from 15 virgin female CRG (3–13 days old) on an SE-30 capillary column

Statistics

Using the Levene test for homogeneity of variance, data sets were tested for homogeneity of variance and log-transformed ($x + 1$) as necessary. Data were analyzed using a one-way analysis of variance, F at $P < 0.05$, with *post hoc* comparisons using Fisher's least significant difference (LSD) test.

Results and Discussion

Pheromone Identification

Coupled GC-EAD analyses of female effluvia extracts demonstrated that male beetle antenna consistently responded to two compounds (Fig. 1B). Two EAD-active peaks from 12 female effluvia collections were observed at 10.39 and 11.14 min on a 30-m SE-30 capillary column and at 10.82 and 14.27 min on a 30-m Stabilwax column at about a 1:3 ratio. The MS of the active component corresponding to the later EAD response in the effluvia extracts exhibited a comparatively strong ion at *m*/*z* 222 (5%) as the highest mass fragment and matched spectra of monounsaturated C_{16} derivatives retrieved from the Wiley 275 mass spectral database. In addition, the MS of the earlier component showed the largest fragment at *m*/*z* 220 (3%), 2 amu less than the later compound, indicating that it could possess an additional unsaturation.

The identity of the EAD-active compounds was determined by capillary GC-MS analysis of DMDS derivatives of the female effluvia extracts. Pairs of diagnostic sulfide fragments were observed at *m*/*z* 161 (81%) and 173 (100%) with the molecular ion at *m*/*z* 334 (52%). Pairs of diagnostic fragments were also observed at *m*/*z* 159 (18%) and 173 (100%) with molecular ion at *m*/*z* 332 (44%). These observations indicated that Δ7-16:OH and Δ7-16:Ald, respectively, were likely candidates for the natural pheromone. The Δ7-16:OH was also verified by a pair of diagnostic sulfide fragments observed at *m*/*z* 173 (94%)

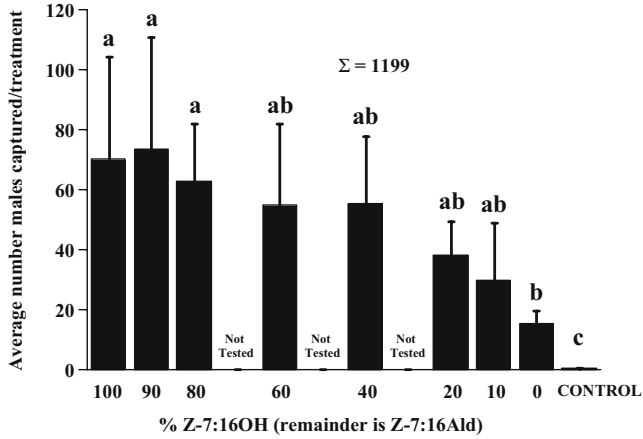

Fig. 2 Average capture/treatment (mean ± SE) of male CRG beetles in traps baited with various 1000-μg blends of Z7-16:OH and Z7-16:Ald, 1999. Data were transformed using log($x + 1$) before analysis. Bars with the same letter are not significantly different ($P < 0.05$, Fisher's LSD test)

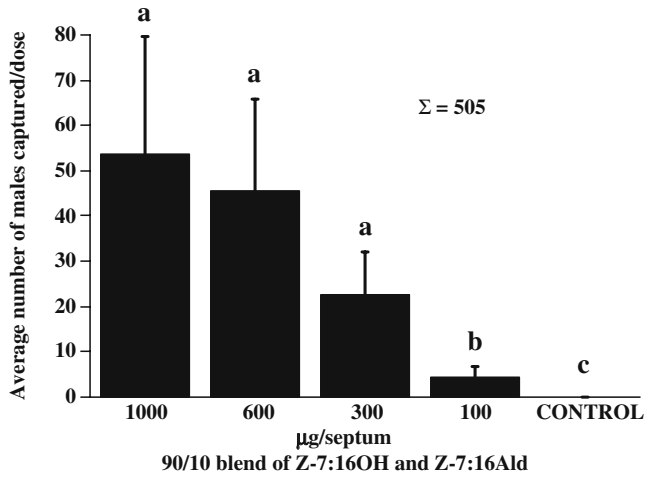

Fig. 3 Average capture/treatment (mean ± SE) of male CRG beetles in traps baited with various doses of a 90:10 blend of Z7-16:OH and Z7-16:Ald, 2000. Data were transformed using $\log(x + 1)$ before analysis. Bars with the same letter are not significantly different ($P < 0.05$, Fisher's LSD test)

and 203 (75%) with the molecular ion at *m*/*z* 376 (30%) from the microacetylation preparation of the female effluvia collection. To confirm the above conclusion, and to determine pheromone geometry, synthetic standards of the (*E*) and (*Z*) isomers of Δ7-16:OH, Δ7-16:Ald, and Δ7-16:Ac were then subjected to the corresponding analysis. The MS spectra and GC retention times of synthetic Z7-16:OH, Z7-16:Ald, Z7-16:Ac, and their DMDS adducts were indistinguishable from those of natural products on both SE-30 and Stabilwax capillary columns. The natural pheromone in each case corresponded to the later-eluting isomers (monounsaturated C_{16} derivatives) with an SE-30 capillary column and earlier-eluting isomers (DMDS adducts) on a Stabilwax capillary column, which established

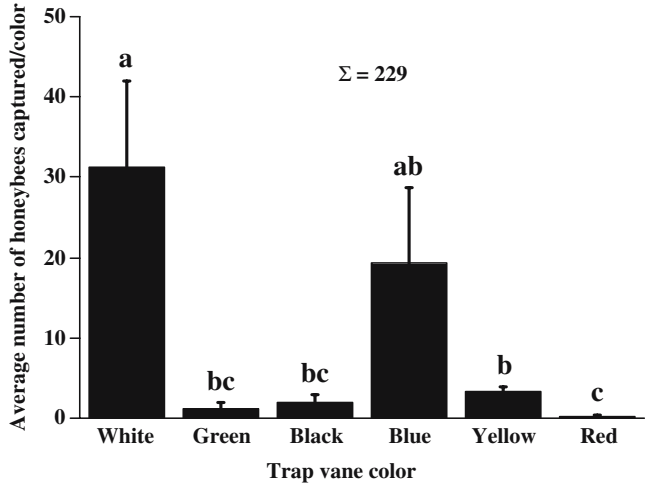

Fig. 4 Average capture/treatment (mean ± SE) of honeybees in traps of various vane colors, 2000. Bars with the same letter are not significantly different ($P < 0.05$, Fisher's LSD test)

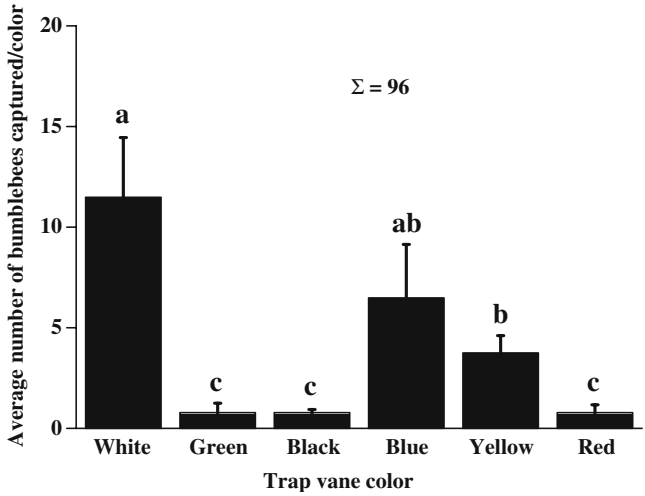

Fig. 5 Average capture/treatment (mean ± SE) of bumblebees in traps of various vane colors, 2000. Data were transformed using log(x + 1) before analysis. Bars with the same letter are not significantly different (P < 0.05, Fisher's LSD test).

these components to be the (*Z*) isomers. The strong antennal responses to Z7-16:OH and Z7-16:Ald were confirmed with authentic standards (Fig. 1A).

Effect of Different Proportions of the Pheromone Constituents on CRG Trap Catches

Traps baited with Z7-16:OH only captured significantly more males than did traps baited with Z7-16:Ald. Male captures decreased steadily in response to increasing amounts of Z7-16:Ald relative to the Z7-16:OH, but in all cases, baited treatments captured more males than the control ($F_{8,18}$ = 8.18; P < 0.001; Fig. 2).

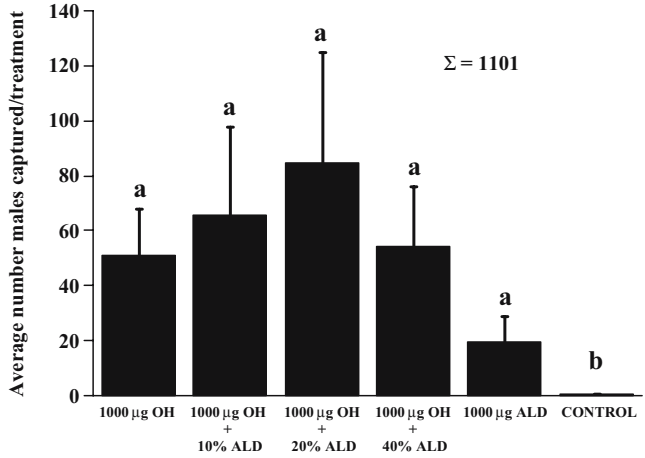

Fig. 6 Average capture/treatment (mean ± SE) of male CRG beetles in traps baited with blends of Z7-16:OH and Z7-16:Ald, 2004. In treatments containing Z7-16:OH, the Z7-16:OH was held constant at 1000 µg/septum. Data were transformed using log(x + 1) before analysis. Bars with the same letter are not significantly different (P < 0.05, Fisher's LSD test).

Effect of Different Doses of a 90:10 Blend of the Alcohol and Aldehyde on Trap Catches

Traps baited with the 100-μg dose captured a smaller number of males than did traps baited with the 300, 600, or 1000-μg doses ($F_{4,15} = 13.32$; $P < 0.001$; Fig. 3).

Effect of Trap Vane Color on CRG and Nontarget Insect Catches

In the 2000 vane color trial, color of the trap vanes did not affect the number of males captured when traps were baited with lures loaded with 1000 μg of a 90:10 ratio of Z7-16:OH and Z7-16:Ald ($F_{5,18} = 0.35$; $P = 0.87$; data not shown). However, the numbers of honeybees ($F_{5,18} = 4.67$, $P < 0.05$; Fig. 4) and bumblebees ($F_{5,18} = 11.64$, $P < 0.001$; Fig. 5) caught were affected by trap color, being captured most frequently in traps with white or blue vanes. These findings are similar to those reported for *Hoplia equina* LeConte, another scarab pest of cranberry (Weber et al., 2005). Based on the findings of these two studies, it is recommended that green or black vanes be used, thereby ensuring effective male capture while reducing the negative impact on beneficial insects.

Effect of the Presence of Different Amounts of the Aldehyde on the Optimum Dose of the Alcohol on Male Catches

In 2004, there were no significant differences among baited treatments ($F_{5,18} = 5.98$, $P < 0.05$; Fig. 6). The high averages in the treatments testing 10% Z7-16:Ald and 20% Z7-16:Ald were caused by two large capture events that grossly inflated the averages in those treatments. In 2005, there were no significant differences among the treatments ($F_{2,11} = 0.096$, $P = 0.91$; data not shown).

Although the female-produced sex pheromone of the CRG contains both Z7-16:OH and Z7-16:Ald, and each compound alone captures males, the Z7-16:Ald, when combined in various ratios with the Z7-16:OH, did not increase male capture. We conclude that although both compounds can be classified as pheromone components, Z7-16:OH alone is sufficient for monitoring or management programs.

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